

Validation of the Global Allergy and Asthma European Network (GA²LEN) chamber for trials in allergy: Innovation of a mobile allergen exposure chamber

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Background: Field clinical trials of pollen allergy are affected by the impossibility of predicting and determining individual allergen exposure because of many factors (eg, pollen season, atmospheric

variations, pollutants, and lifestyles). Environmental exposure chambers, delivering a fixed amount of allergen in a controlled environmental setting, can overcome these limitations.

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Environmental exposure chambers are currently already used in phase 2, 3, and even 4 trials. Unfortunately, few chambers exist in the world, and this makes it difficult to perform large, multicenter clinical trials. The new Global Allergy and Asthma European Network (GA²LEN) mobile exposure chamber is a step forward because the mobility of the chamber makes it convenient for patients to participate in clinical testing.

Objective: This study was made to validate the reproducibility, sensitivity, and specificity of the results obtained in the new GA²LEN chamber.

Methods: Seventy-two adult patients (19–61 years old) with allergic rhinitis with or without asthma caused by grass pollen were included in different clinical validation tests. Total symptom scores and total nasal symptom scores were recorded at time zero (0) and every 10 minutes during exposures, along with nasal and respiratory parameters.

Results: Exposure tests confirmed the reproducibility between subsequent runs and the sensitivity ($P < .00001$ vs patients exposed to placebo) and specificity (very low score in nonallergic subjects) in the GA²LEN chamber. No adverse reactions were recorded during the tests.

Conclusions: The mobility of the GA²LEN chamber provides a new, potentially effective, and safe way of generating reliable data in allergy multicenter clinical trials. (J Allergy Clin Immunol 2016;■■■:■■■–■■■.)

Key words: Allergy trial, environmental exposure chamber, validation, grasses, placebo, Global Allergy and Asthma European Network (GA²LEN)

Field allergy clinical trials depend on natural pollen exposure and are therefore biased by a number of factors, the most important being the variability of the pollen seasons, the changing environmental conditions, and the uncertainty in determining the actual individual exposure.^{1–3} This is especially true in multicenter trials, in which pollen counts and environmental conditions can be very different from one site to another. These aspects are well summarized by Bousquet et al.⁴ In multicenter studies there is also a risk of excluding some patients from the final analysis because of the lack of relevant pollen exposure in one area.⁵

These problems can be overcome by the use of environmental exposure chambers (EECs).^{2,6,7} Currently, however, EECs are accepted by the European Medicines Agency and US Food and Drug Administration only for phase 2 dose-ranging studies,⁸ even though the use of EEC facilitates proper control and standardization of the quantitative allergen exposure. Further advantages include shortening the time frame of the dose-finding process, avoiding the confounding effects of rescue medications, and using smaller numbers of subjects than would be required in natural exposure studies.⁶ EECs have been previously used for evaluating the efficacy of pharmacotherapies for seasonal allergic rhinitis,^{9–11} both in phase 2 and still ongoing in phase 3 and even phase 4 trials,¹² and studies have also used EECs in allergen immunotherapy (AIT) investigations.^{13–15} A recent position paper from the European Academy of Allergy and Clinical Immunology states that “EEC is likely to be a good option as an adjunct to natural exposure studies for phase 3 Randomized Controlled Trials” also in AIT studies.¹⁶

However, there are drawbacks to the existing stationary EECs. Very few such facilities exist around the world,³ and this makes it very difficult to perform multicenter clinical trials.⁷ The reproducibility of allergen exposure and treatment outcomes between

Abbreviations used

AIT:	Allergen immunotherapy
AUC:	Area under the curve
EEC:	Environmental exposure chamber
GA ² LEN:	Global Allergy and Asthma European Network
PDU:	Particle dispersal unit
PNIF:	Peak nasal inspiratory flow
TNSS:	Total nasal symptom score
TSS:	Total symptom score
VAS:	Visual analog scale

different EECs is also a potential problem.¹⁷ Furthermore, pollen in the stationary EECs is reventilated by a strong airflow, prohibiting measurement of individual exposures or a placebo control within a run and being potentially irritating for the eyes and nose.

The Global Allergy and Asthma European Network (GA²LEN) has developed a novel mobile EEC called the GA²LEN chamber to overcome these problems. The new mobile GA²LEN chamber has been technically and clinically validated according to current needs and requirements, as described in the article by Rosner-Friese et al.³

METHODS

Technical validation

The GA²LEN chamber consists of 2 standard 24-foot-high cubic containers forming a compound of 7.45 m × 4.90 m × 2.86 m. One container accommodates an office and changing room (A), and the other (B) houses the test chamber itself and the installations for air conditioning (Fig 1). When set up, the containers are interlocked, forming an interconnected compound, allowing access to the test chamber through the changing room. Even though the GA²LEN chamber consists of 2 containers, it can be transported by a single truck equipped with a crane. Minimal requirements are necessary for the onsite setup, which requires only external electricity (or a generator), to ensure the GA²LEN chamber can be operated at a maximum number of trial sites. The test chamber can accommodate up to 9 patients plus a nurse or a doctor, providing chairs that are recommended for a seating time of up to 12 hours, with adjustable footrests and surfaces that can be sanitized. Each chair has its own tablet holder. The chairs are at fixed positions but can be removed for full cleaning (Fig 2).

The airflow in the test chamber was designed to be as low as possible, meaning not recognizable, at each seating position to not create any adverse reactions because of airflow (eg, feeling of nasal obstruction or eye reddening) but still create sufficient air exchange to keep CO₂ and O₂ at comfortable levels. Nevertheless, the air conditioning installed allows the chamber temperature to be adjustable from 15°C to 35°C, whereas relative humidity levels can be set from 30% to 80% (noncondensing). Gas levels, namely oxygen, carbon dioxide, carbon monoxide, and ozone, are constantly monitored and recorded. The outside air is filtered by using a multistage filter system, removing more than 99% of particles smaller than 1 μm (and all allergens) from the air.

Because of the air conditioning capability and the extensive insulation, experiments can be performed at outside temperatures of –10°C to 35°C, whereas outside relative humidity can be handled from 20% to 80%. The test chamber is monitored by cameras and microphones. This allows doctors and nurses to also monitor the patients remotely throughout the test procedure while being located in the office room of the GA²LEN chamber. If agreed to by signed consent, visual and audio data are also recorded. These recordings can be used to validate symptom scores, such as number of sneezes.

Because of the limited seating available in the exposure chamber, a fast turnaround time, meaning the time from ending one test until being able to start another test, is necessary to enable several test runs within a day. The major issue contributing to the turnaround time is the necessity to run the



FIG 1. The GA²LEN mobile exposure chamber.

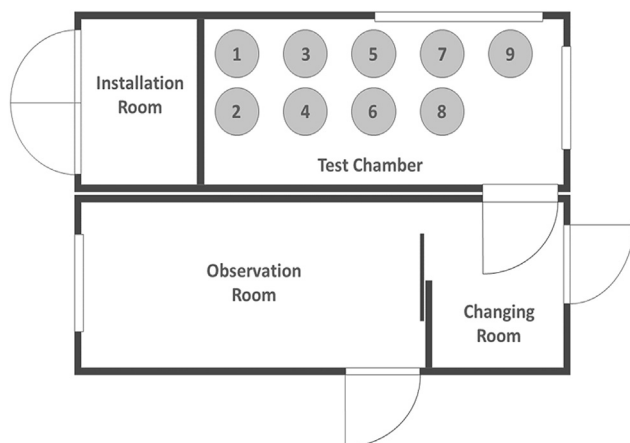


FIG 2. The GA²LEN mobile exposure chamber: interior layout. Numbers refer to seats for patients.

cleaning protocol after each test, which ensures equal conditions for each test run. To reduce the cleaning time, the GA²LEN chamber is not flushed with pollen, which would result in pollen sticking to every surface in the room, including the ceiling and walls. In the GA²LEN chamber pollen is dispersed only where the subjects are seated, and the chamber airflow is designed so that no other surfaces can be reached by the pollen, except the subjects' chairs, tablet holders, the floor directly beneath the subjects, and the subjects themselves. Therefore the subjects are required to wear clean-room apparel to minimize the amount of pollen adhering to the patient when leaving the chamber. Wearing clean-room apparel also prevents other aeroallergens from being introduced into the chamber. The floor is laid out as a double flooring, and therefore pollen reaching the floor will fall through a metal grid, which makes up the upper floor. Pollen falling through the grid will then be either sucked through the exhaust air outlet and then removed by filtering the air or will stick to the lower surface. The airflow design in the chamber prevents the pollen from refloating. Not only does this approach allow for faster back-to-back

testing, it also allows for individualized testing, especially for research purposes, where there are smaller cohorts but multiple differently parametrized exposures. The GA²LEN chamber dispersal system is designed to have a dedicated particle dispersal unit (PDU) placed above each chair, with its nozzle positioned in a way that the particle concentration in the breathable air of a patient seated underneath is constant in a distinct region. This region is thoroughly confined, so that the particles dispersed from 1 PDU are not able to be inhaled by a patient at another seat.

The height of the chair is adjusted when a patient is seated, so that the nostrils of the patient are at a defined level, which is called the calibrated exposure height. Breathable air is defined as the 2 L of air surrounding the upper airway organs during each breath intake. Because the patient can move around (not by moving the chair but by moving the upper body), the breathable air is actually defined as an ellipse of 40 cm × 30 cm around the nose and mouth at calibrated exposure height. The PDU takes particles from a blister and disperses these particles through a nozzle. A blister in this case is made of 2 sealed stripes with a single-layer (height) particle line in between. The width of the line is controlled with narrow margins in the blistering process. Before dispersal, each particle is photographed and counted. Each image is stored for later quality assurance tasks. Because of previous individually performed calibrations, the defined and required particle concentration in the patient's breathable air can be controlled easily by the speed at which the blister is emptied. A PDU can generate a particle concentration from 100 particles per cubic meter of breathable air up to 15,000 particles per cubic meter during a time frame of up to 8 hours.

Clinical validation

Patients and methods. All tests were performed in Berlin, Germany, outside the relevant pollen season by using *Phleum pratense* (Allergon AB, Ängelholm, Sweden) as the grass species. Reproducibility tests were also performed after the chamber had been disassembled and then transported to Barcelona and back. Based on power analysis, altogether, 72 adult patients (19-61 years), both male and female (40% and 60%, respectively), were included in the different validation tests.

Inclusion criteria were a history of mild-to-moderate allergic rhinitis with or without asthma caused by grass pollen for 2 or more years, a skin prick test

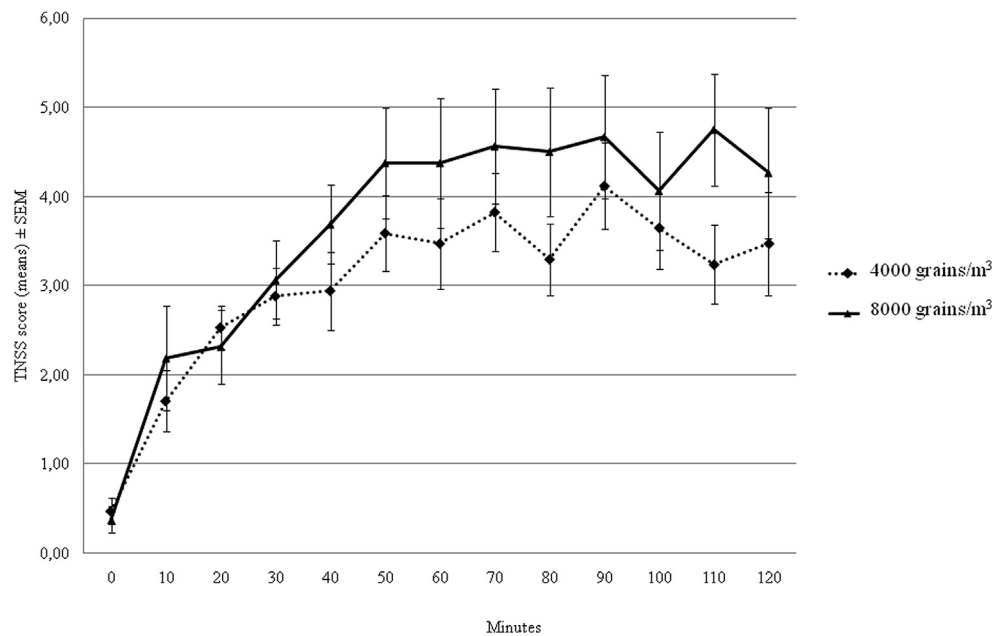


FIG 3. Comparison of response to 4000 and 8000 grass pollen grains/m³ exposure in allergic subjects (n = 16 patients).

response of 3 mm or greater and/or an ImmunoCAP score of 2 or greater, and FEV₁ of 70% of predicted value or greater. Exclusion criteria included previous treatment with AIT, treatment with systemic corticosteroids within the last month, treatment with antihistamines or other drugs with antiallergic properties within the last 3 days, and suspicion of pregnancy. Five control subjects were nonatopic.

Patients were exposed during different lengths of time (90, 120, and 240 minutes) to 2 different pollen concentrations (4000 and 8000 grass pollen grains/m³) and placebo. Because concentrations of 4000 and 8000 grass pollen grains/m³ produced comparable results (Fig 3), we report here only the outcomes of exposure to 4000 grass pollen grains/m³.

Patients recorded eye (itching, foreign body sensation, redness, and watering), nose (itching, sneezing, running, and blocked), and bronchial (wheezing, shortness of breath, and cough) symptoms. For every symptom, a score of 0 to 3 was applied (none, mild, moderate, and severe). The total symptom score (TSS) is the sum of all 3 scores (ie, eye/nose/lung; minimum = 0 and maximum = 36). The total nasal symptom score (TNSS) is the sum of the 4 nasal symptoms (minimum = 0 and maximum = 12). Subjective parameters were recorded at time zero (0) and every 30 minutes during exposure. The visual analog scale (VAS) score, peak nasal inspiratory flow (PNIF), and peak expiratory flow were also recorded before entering the chamber, after acclimatization in the chamber (10-30 minutes, time zero), and every 30 minutes during exposure.

Statistical methods. Primary analysis is based on the area under the curve (AUC) of values based on the per-protocol population. The statistical model is based on an assumption of data having a symmetric distribution (optimally a Gaussian distribution), and hence the log AUC values are expected to be better suited for analysis than the arithmetic AUC values. This was confirmed by means of analysis. Because some AUC values are 0, log (AUC+1) values are used; an approximate back transformation from a log (AUC+1) value of y is presented as follows: $x = (\exp[y] - 1)/120$, where x corresponds to a slightly weighted average of TNSSs over the 13 time points (time 0-120). Aggregated values are analyzed as the values of the logAUC120 calculated by using the traditional trapezoidal formula. The active effects are estimated and tested by using a mixed analysis of covariance model in which covariates include age, sex, baseline score, number of the run, and subjects. All analyses are programmed with SAS version 9.3 (SAS Institute, Cary, NC).

Ethical conduct of the study. The study was approved by the ethics committee of the University Hospital Charité, Berlin, and all patients provided written informed consent according to the Helsinki declaration.

RESULTS

Reproducibility

The reproducibility of symptoms evoked in the EEC is one of the crucial points for clinical validation.³ Eighteen allergic subjects were exposed 2 times in the mobile chambers to 4000 grass pollen grains/m³ for 90 minutes at intervals of 1 to 5 weeks between visits. VAS scores and PNIFs were recorded every 30 minutes. For both VAS scores (average maximum score: first visit = 2.34, second visit = 2.52) and PNIFs (average maximum score: first visit = 136 L/min, second visit = 151 L/min), results were comparable between the 2 exposures (Fig 4). There was no change in pollen distribution and exposure observed after disassembling, moving, and reassembling the chamber.

Comparison between placebo and active run

A total of 101 subjects took part in a placebo and active run. Seventy-seven subjects were exposed to 4000 grass pollen grains/m³ for 120 minutes. Patients with active grass allergy reached a mean \pm SEM maximum TSS of 5.2 ± 0.4 and TNSS of 3.8 ± 0.2 at 70 minutes. By comparison, 24 placebo-treated subjects reached a mean maximum TSS of 2.6 ± 0.4 at 70 minutes and TNSS of 1.7 ± 0.3 at 70 minutes (Fig 5 for TNSS). The differences between TSSs and TNSSs recorded after exposure for 120 minutes to 4000 grass pollen grains/m³ of grass and placebo were highly significant ($P < .00001$). Significant differences were also observed in PNIF values and in nasal secretion between grass-exposed and placebo-exposed subjects ($P < .0001$).

No symptoms in nonatopic subjects

The specificity of the EEC exposure was demonstrated in a test in which 5 nonatopic subjects were exposed for 120 minutes to 4000 grass pollen grains/m³ and compared with 36 allergic patients. Subjects recorded TNSSs, ocular scores, and bronchial

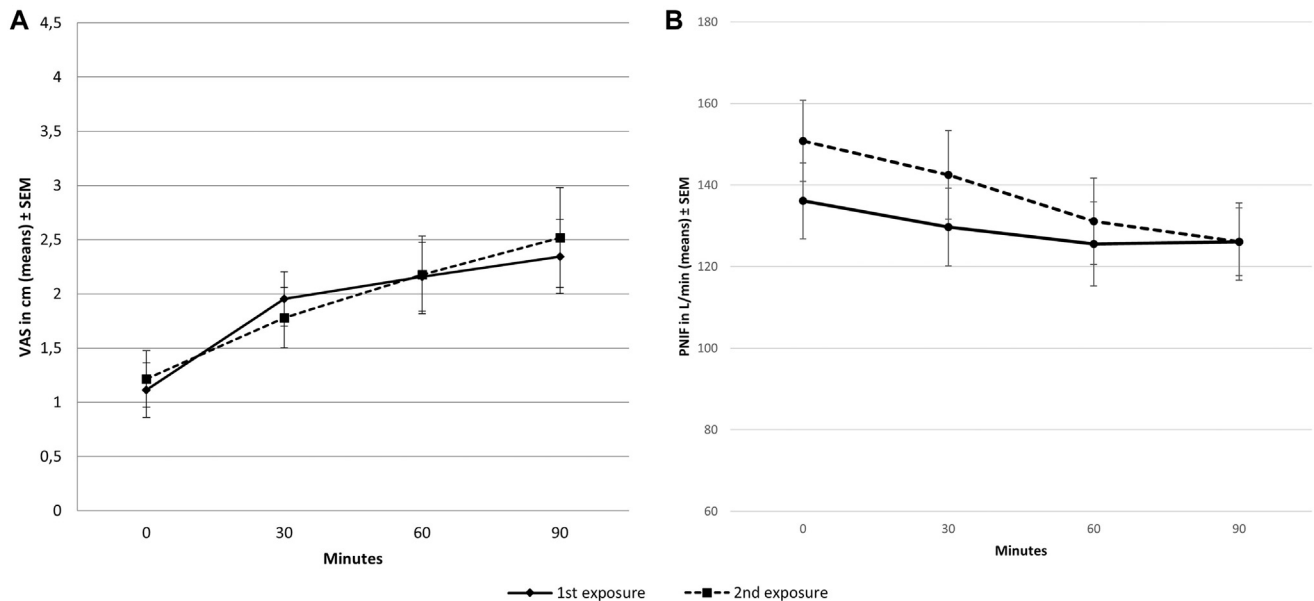


FIG 4. Reproducibility of EEC challenges in subsequent exposures. Eighteen allergic subjects were exposed 2 times to 4000 grass pollen grains/m³ for 90 minutes at intervals of 1 to 5 weeks between visits. **A**, VAS scores. **B**, PNIFs.

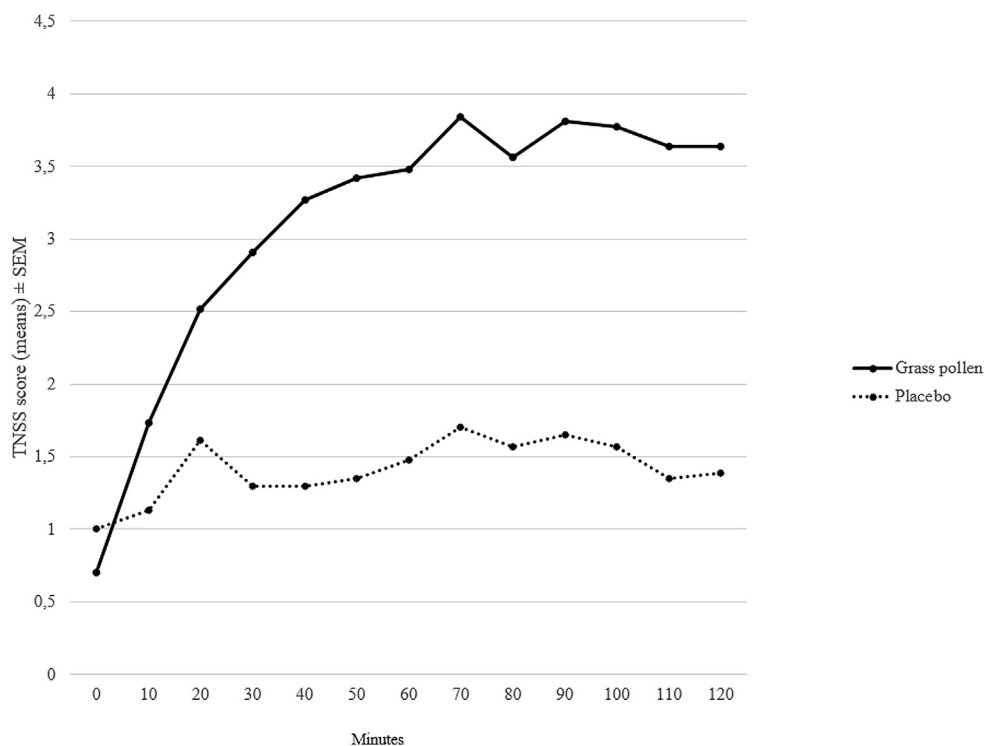


FIG 5. Comparison of response to grass pollen and placebo exposure in allergic subjects (grass pollen, n = 55; placebo, n = 30). Exposure was 4000 grass pollen grains/m³ ($P < .0001$).

scores every 10 minutes, according to the previously described scoring system (minimum = 0, maximum = 36). None of the nonatopic patients reached a TSS of 1 (Fig 6).

No effect of priming

Six patients were exposed for 120 minutes to 4000 grass pollen grains/m³ on 5 consecutive days. TSSs were recorded

every 10 minutes, and VAS scores were recorded every 30 minutes. No difference was detected in either outcome between day 1 and day 5 (Fig 7).

Plateau reached

Seventeen patients were subjected to a longer exposure of up to 240 minutes. A TSS and TNSS plateau was reached after

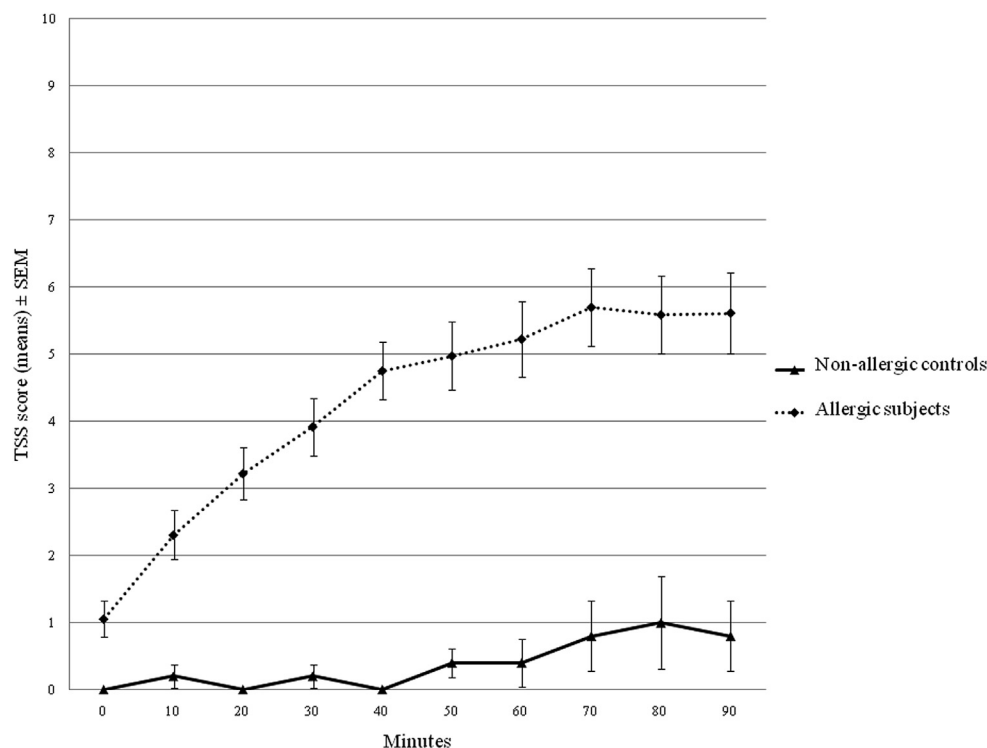


FIG 6. Comparison of response to grass pollen exposure in allergic subjects and nonallergic control subjects ($n = 36$ allergic subjects and 5 nonallergic control subjects). Exposure was 4000 grass pollen grains/ m^3 .

70 minutes, with a peak at 90 minutes and no further increase at up to 240 minutes. Average peak values at 90 minutes were 5.4 (minimum-maximum, 1-14) for TSSs and 4.1 (minimum-maximum, 1-8) for TNSSs (Fig 8).

Safety

All exposure tests were safe and well tolerated. No clinically significant adverse reactions were observed during the EEC exposure, and no rescue medication had to be provided. No late reactions were reported after 24 hours.

DISCUSSION

Clinical tests performed in the new GA²LEN mobile chamber showed high sensitivity, specificity, and reproducibility. Allergic patients had a highly significant response compared with patients exposed to placebo, whereas nonallergic patients showed no response in terms of TNSSs. VAS scores observed in our validation study indicate that patients included in this study could be classified as having “mild-to-moderate rhinitis” according to the Allergic Rhinitis and its Impact on Asthma Guidelines,¹⁸ as stated by Bousquet et al.¹⁹ This is important because the difference from placebo is generally statistically less pronounced than in severely affected patients.²⁰ TNSSs observed in our study are comparable with those reported by Hohlfield et al²¹ with the same scoring system and at the same pollen concentration. A minimum placebo response was observed in the placebo-exposed patients. This has been observed before,³ and it is well known that the placebo effect is particularly relevant in allergy trials.^{22,23}

To avoid subjective biases, we assessed the reproducibility in the GA²LEN chamber with subjective and objective parameters, VAS scores and PNIF values, in 2 subsequent visits.²⁴ VAS scores have been found to correlate with disease severity and to detect the variations of symptoms in patients with allergic rhinitis with high sensitivity.^{25,26} PNIF measurement is a very effective tool for assessing the severity of congestion in allergic rhinitis,^{27,28} and nasal flow has also been evaluated by Krug et al²⁴ for validation of the Fraunhofer chamber. In our study both VAS scores and PNIFs showed comparable results between the 2 subsequent exposures. Similar results have been observed in the past with stationary chambers. Hohlfield et al²¹ exposed 60 patients with grass pollen allergy to 4000 grass pollen grains/ m^3 in the Fraunhofer ECC on 2 occasions during the pollen season, with 2-week intervals. TNSSs were highly reproducible between the 2 exposures.²¹ Interestingly, VAS scores reported by Hohlfield et al²¹ at the end of a 4-hour exposure are comparable with VAS scores observed in our study and were also highly reproducible.

Krug et al,²⁴ still using the Fraunhofer ECC, found comparable results between 5 consecutive exposures evaluating TNSSs, nasal flow rate, nasal secretion, FEV₁, and peak expiratory flow, with a grass pollen concentration of 8000 grass pollen grains/ m^3 for 4 hours. In this study a significant but not clinically relevant reduction was observed in the mean FEV₁, indicating a priming effect.²¹

All these results confirm that symptoms elicited in consecutive exposures in the GA²LEN mobile chamber have the same reproducibility of symptoms provoked in the classic stationary EECs. In contrast to previous reports,^{3,24,29} we did not observe any priming effect, with objective end points in 6 patients challenged on 5 consecutive days. This can be due to either the small number of

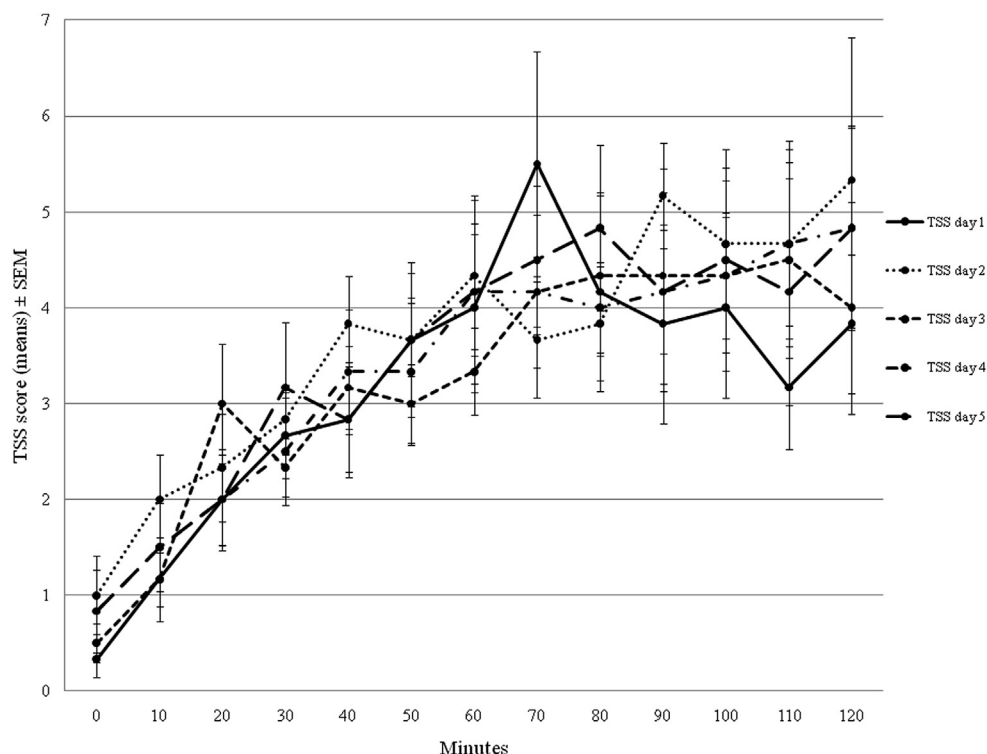


FIG 7. No evidence of priming effect in 5 exposures in consecutive days ($n = 6$ patients). Exposure was 4000 grass pollen grains/ m^3 .

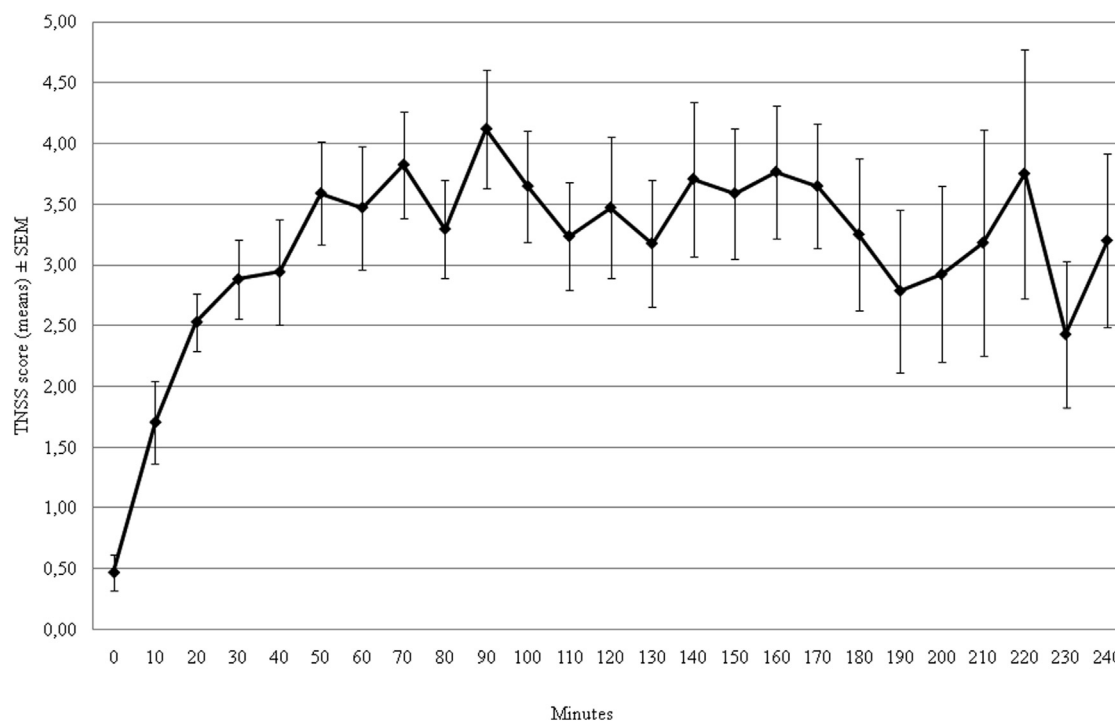


FIG 8. No symptom increase after prolonged exposure ($n = 17$ patients). Exposure was 4000 grass pollen grains/ m^3 .

studied patients, the shorter exposure, or lower pollen grains exposure compared with the study by Krug et al²⁴ (2 vs 4 hours; 4000 vs 8000 grass pollen grains/ m^3) or to the lack of a seasonal exposure, which has been deemed relevant in previous publications.^{6,29}

One of the key questions regarding EECs is whether symptoms evoked in the chambers are comparable with those observed during seasonal real-life exposure.^{3,24,30} Two studies addressed this particular point. Jacobs et al³¹ found a strong correlation between nasal and ocular symptoms reported during natural

ragweed exposure and those evoked in an EEC. By contrast, Hohlfield et al²¹ found no correlation between the TNSS evoked at the end of a 4-hour grass pollen exposure in the EEC and symptoms registered during the pollen season. However, a good correlation was found with the TNSS registered 24 hours after the challenge. The authors concluded that TNSSs after 24 hours better reflect the late-phase reactions occurring during natural pollen exposure.¹⁹ Correlation between the symptoms in the chamber and natural allergen exposure suggests that EECs can be used as a surrogate for the natural allergy season exposure in clinical trials.¹⁷ Field pollen allergy clinical trials suffer from some biases, the most important being the variability of the real allergen exposure of every study site and every single subject included in the studies.^{3,7} Seasonal and local variations (humidity, temperature, local specific pollutants, and different concentrations of major allergens in the pollen) can have a big influence on the pollen counts and symptoms. Furthermore, real exposure heavily depends on the lifestyle of the subjects (indoor/outdoor) and indoor environmental exposures.^{1,3}

Another problem is the difficulty of correctly defining the pollen season, with its peak and duration being very different from one year to another and differing by locations.⁷

Furthermore, the classic inclusion criteria in clinical trials (skin tests, allergen-specific IgE, and clinical history) are not able to distinguish patients with different levels of symptoms, leading to the inclusion of patients with very low symptom scores, another possible bias cause.^{30,31} Screening in an EEC, as done previously,⁶ could overcome this issue by guaranteeing minimal symptoms with exposure according to entry criteria.^{1,2,17}

For these reasons, although the European Medicines Agency already accepts the use of provocation tests (nasal, bronchial, conjunctival, or exposure chambers) for phase 2 and dose-finding studies in AIT,⁸ the already mentioned European Academy of Allergy and Clinical Immunology position paper on clinical outcomes in AIT considers the EEC a promising vehicle for phase 3 clinical trials in AIT.¹⁶ Actually, a number of controlled phase 3 and 4 studies are ongoing with antiallergic drugs using exposure chambers.¹² Compared with traditional provocation tests, the EEC allows testing of many patients at the same time in a more natural way and, above all, records all of the elicited organ symptoms, as happens in natural exposure.

Furthermore, there are also important financial issues. The high costs of drug development are mainly based on the costs of the clinical trials, and shorter trials are needed. The novel EEC technology has a potential to perform trials more efficiently than field trials because less patients need to be included under standardized conditions. Unfortunately, very few such chambers exist in the world today, and comparability studies of individual EEC facilities are lacking because of the great geographic distances between individual pollen chambers and the specific technical features of each EEC facility.^{16,17}

These issues make the required multicenter clinical studies very difficult to perform. Thus the mobile GA²LEN chamber represents a unique and novel solution to explore the biology of diseases of the airways and potential therapeutic interventions using multiple clinical trial sites to ensure the diversity of populations necessary to better define study end points.

Clinical implications: EECs are increasingly used in allergy studies but have some limitations. The use of a mobile exposure chamber can represent an advantage in clinical studies of AIT and pharmacotherapy.

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